

Amendments to the Specification

Please replace the paragraph beginning at page 1, line 9, with the following amended paragraph:

BACKGROUND OF THE INVENTION FIELD OF THE INVENTION

1. Field of the Invention

The present invention relates to a composition comprising an extract of the *Aphanizomenon*[[-]] *flos-aquae* var. *flos-aquae* alga, which may be applied topically. It applies more particularly but not exclusively, to the treatment of the upper layers of the epidermis and/or of hair, notably for preventing and treating induced skin ageing and/or for repairing certain changes in skin tissues such as stretch marks and/or for contributing to improving the hair's aspect.

Please insert the following section heading at page 1, between lines 14-15:

BACKGROUND OF THE INVENTION

Please replace the paragraph beginning at page 1, line 29, with the following amended paragraph:

The present invention relates to the use of a unique variety of cyanobacteria, a variety discovered in Lake Klamath, Oregon (USA) and characterized by Renhui et al. (Renhui Li, Wayne W. Carmichael, Yongding Lui & Makoto M. Watanabe, Hydrobiology, 438: pages 99-105, 2000, Taxonomic reevaluation of *Aphanizomenon*[[-]] *flos-aquae* NH-5 based on morphology and 16S rRNA gene sequences).

**Please replace the paragraph beginning at page 2, line 11, with the following amended paragraph:**

Preparations based on dried *Aphanizomenon*[-] *flos-aquae* var. *flos-aquae* are recommended as a food complement for their numerous constituents, notably their high content in highly assimilable proteins and the presence of vitamins B6, B12 and F.

**Please replace the paragraph beginning at page 2, line 15, with the following amended paragraph:**

Indeed, the listed investigations show that oral administration of *Aphanizomenon*[-] *flos-aquae* var. *flos-aquae*:

- allows an increase in the reactivity of the immune system by increasing the synthesis of messenger RNA coding for interleukine 1 (IL-1) (Characterization of human monocyte activation by a water soluble preparation of *Aphanizomenon*[-] *flos-aquae*, *Phytomedicine*, Pugh N, Pasco DS, 2001, Nov. 8(6) : pages 445-53),
- is beneficial to health by the diversity of the nutrients which compose it (Microalgae as food & supplement, Kay RA, *Crit. Rev. Food Sci. Nut.*, 1991, 30(6) : pages 555-73),
- is a good nutritional source of polyunsaturated fatty acids which give it a hypocholesterolemic property (Rafial I. Kushak, Christian Drapeau, Elisabeth M. Van Cott, Harland H; Winter, *JANA*, vol. 2(3), 2000, pages 59-65).

**Please replace the paragraph beginning at page 2, line 30, with the following amended paragraph:**

On the other hand, no document refers to the use of *Aphanizomenon*~~[[—]]~~ *flos-aquae* var. *flos-aquae* in the ~~prerparation~~ preparation of beneficial compositions for preventing skin ageing and improving hair aspect, notably for a topical application.

**Please insert the following section heading at page 3, between lines 7-8:**

#### SUMMARY OF THE INVENTION

Therefore, the object of the invention is to solve these drawbacks by developing a topically applicable composition which allows the active ingredients of *Aphanizomenon*~~[[—]]~~ *flos-aquae* var. *flos-aquae* to be retained in all their integrity so as to be actively involved in treating the upper layers of the epidermis and/or of hair, notably for preventing skin ageing and improving hair aspect.

**Please replace the paragraph beginning at page 3, line 14, with the following amended paragraph:**

For this purpose, it proposes a topically applicable composition comprising at least one extract of *Aphanizomenon*~~[[—]]~~ *flos-aquae* var. *flos-aquae* at a concentration between 0.01 and 10% by dry weight relatively to the total weight of the composition.

**Please replace the paragraph beginning at page 3, line 18, with the following amended paragraph:**

Advantageously, a method for preparing said composition comprising at least one extract of *Aphanizomenon*~~[[—]]~~ *flos-aquae* var. *flos-aquae* comprises the preparation of said extract by extracting active substances

contained in for example dry, dried freeze-dried *Aphanizomenon*[-] *flos-aquae flos-aquae var.* notably according to the following steps:

- at least one maceration at a temperature from 25 to 50°C of dried blue algae of the *Aphanizomenon*[-] *flos-aquae var.* *flos-aquae* species in the presence of enzymes such as cellulases, pectinases and glucanases for a time from ten minutes to ten hours under stirring,
- a liquid/solid separation by centrifugation,
- a liquid/liquid separation by a membrane filtration method,
- drying and/or dilution in a solution containing specific adjuvants, for example sorbitol,
- an optional specific separation of the different thereby extracted constituents for example by chromatography, the different obtained substances able to be used either alone or as a mixture, according to the sought-after effect.

**Please replace the paragraph beginning at page 3, line 14, with the following amended paragraph:**

The unique figure is the illustration of a diagram of a profile obtained by hybridization of complementary DNA probes, marked with different mRNAs obtained with a normal human epidermis treated with a raw aqueous extract of *Aphanizomenon*[-] *flos-aquae var.* *flos-aquae*.

**Please replace the paragraph beginning at page 4, line 27, with the following amended paragraph:**

The method for preparing a composition comprising at least one extract of *Aphanizomenon*[-] *flos-aquae flos-aquae var.* containing active substances comprises the preparation of said extract according to the following steps:

- at least one maceration at a temperature from 25 to 50°C and preferably 35°C, of dried blue *Aphanizomenon* *[-]* *flos-aquae* *var.* *flos-aquae* algae in the presence of cellulases, pectinases, and glucanases for a time from ten minutes to ten hours, and preferably four hours under stirring. The results of the tests show that the attack by the different enzymes provides better solubilization of the parenchymatic wall of the algae and thus a higher polysaccharide richness of the thereby prepared aqueous extract.
- a liquid/solid separation by centrifugation under an acceleration from 5,000 to 10,000 g, and preferably 9,000 g.
- a liquid/liquid separation by a membrane filtration method with a cutoff threshold between 100,000 Daltons and 0.2 µm.
- drying and/or dilution in an aqueous solution of sorbitol.  
By drying, is meant both standard drying (heat) and drying by nebulization or freeze-drying.
- specific separation of the different constituents, thereby extracted by chromatography, the different obtained substances being used alone or as a mixture according to the sought-after effect.

**Please replace the paragraph beginning at page 5,  
line 24, with the following amended paragraph:**

The high density filter or cDNA macroarray method on a support comprising at least 600 characteristic genes of the skin and pilous system was used for investigating the effect of the *Aphanizomenon* *[-]* *flos-aquae* *var.* *flos-aquae* extract

on the expression of genes coding for major proteins of cosmetic or dermo-cosmetic interest.

**Please replace the paragraph beginning at page 6, line 9, with the following amended paragraph:**

The *Aphanizomenon* [-] *flos-aquae* var. *flos-aquae* extract was applied on the explants in an amount of 5 mg/cm<sup>2</sup> of a 2% raw aqueous extract solution without any adjuvant, mornings and evenings for two days.

**Please replace the paragraph beginning at page 6, line 16, with the following amended paragraph:**

The skin pieces (epidermises) were rinsed and then placed in the presence of ~~Tri-reagent~~ TRI-REAGENT® (Sigma T9424) and then frozen at -80°C.

**Please replace the paragraph beginning at page 7, line 9, with the following amended paragraph:**

Membranes of the Custom ATLAS BA 600/1 type are pretreated and then the cDNAs immobilized on each membrane are hybridized (68°C, 12 hours) with corresponding marked probes, the filters are then washed and analyzed by direct quantification of the radioactivity of the spots by means of a phosphorimager PHOSPHORIMAGER® (Cyclone, Packard Instrument) type of apparatus and its QuantArray QUANT ARRAY® (Packard) software.

**Please replace the paragraph beginning at page 7, line 15, with the following amended paragraph:**

Table I below shows the genes, the relative expression (RE) of which was significantly changed after forty-eight hours of a bidaily application of a raw extract of

*Aphanizomenon* **[-]** *flos-aquae* var. *flos-aquae* on a normal human epidermis.

Table I:

	Control	<i>Aphanizomenon</i> <b>[-]</b> <i>flos-</i> <i>aquae</i> <u>var.</u> <i>flos-aquae</i> extract	
Name of the genes	RE	RE	%
Vimentin (VIM)	10.0	21.8	217
Metalloprotease 11 (MMP11)			
Stromelysine 3	6.2	15.9	255
Metalloprotease 3 (MMP3) ; Stromelysine 1 (STMY1; SL1) ; Transin 1	5.7	17.3	306
Tissular inhibitor of metalloprotease 1 (TIMP1) ; Erythroid potentiator activity (EPA) ; Inhibitor of fibroblastic collagenases	10.0	24.3	244
Gamma sub-unit of the interleukine-2 receptor (IL-2R gamma; IL2RG) ; Common receptor of gamma chains of cytokines; P64	6.6	20.0	302
Epidermal filaggrin (FLG)	28.7	7.3	25
Loricrin (LOR; LRN)	31.9	11.7	37
Protein related to differentiation of adipocytes	15.8	23.1	146
Beta integrin (ITGB4) ;	24.7	40.1	162

antigen CD104			
S100 A7 protein binding calcium; psoriasin	135.2	202.0	149
S100 A8 protein binding calcium (S100A8); Calgranulin A (CALA); Migration inhibitory factor- related protein 8 (MRP8); Leukocyte L1 complex light chain; Cystic fibrosis antigen (CFAG)	311.1	485.4	156
S100 A9 protein binding calcium (S100A9); Calgranulin B (CAGB); Migration inhibitory factor- related protein 14 (MRP14); Leukocyte L1 complex light chain;	210.2	323.8	154
Ornithine decarboxylase (ODC)	9.4	18.1	193
Spermidine acetyltransferase	13.9	26,3	189
Elafin; specific inhibitor of elastases (ESI); skin-derived antileukoproteinase (SKLP)	22.6	34.3	152
Calmodulin-like skin protein (CLSP)	31.8	16.0	50

Please replace the paragraph beginning at page 9,  
line 2, with the following amended paragraph:

The diagram of the unique figure illustrates the  
profile obtained by hybridization of complementary DNA probes  
marked with different mRNAs obtained with normal human

epidermis treated with a raw aqueous extract of  
*Aphanizomenon*[-]*flos-aquae* var. *flos-aquae*.

**Please replace the paragraph beginning at page 9,  
line 2, with the following amended paragraph:**

The treatment of skin explants by an extract of  
*Aphanizomenon*[-] *flos-aquae* var. *flos-aquae* induces  
significant changes in the expression of the differentiation  
and proliferation of cells of the epidermis. These changes are  
identical with those obtained with a compound of the retinol  
(or retinoid: lipid which directly diffuses into the plasmic  
membrane) type without the *Aphanizomenon*[-] *flos-aquae* var.  
*flos-aquae* extract having the formulation constraints.

**Please replace the paragraph beginning at page 10,  
line 12, with the following amended paragraph:**

The raw extract of *Aphanizomenon*[-] *flos-aquae*  
var. *flos-aquae* reduces the expression of CLSP, twice; this is  
an argument in favor of its involvement in the modulation of  
this marker.

**Please replace the paragraph beginning at page 10,  
line 15, with the following amended paragraph:**

Moreover retinoids inhibit the expression of  
loricrin (Brown L.J. et al., Retinoic acid suppression of  
loricrin expression in reconstituted human skin cultured at  
the liquid-air interface, J. Invest. Dermatol., 1994 June,  
102(6), 886-90), as well as the raw extract of  
*Aphanizomenon*[-] *flos-aquae* var. *flos-aquae* which reduces  
its expression by more than 10 fold.

Please replace the paragraph beginning at page 10, line 27, with the following amended paragraph:

Loricrin is the major constituent of the wall of corneocytes, and is contained in the granules up to the terminal stage of the differentiation and then contributes to the formation of the envelope of the corneocytes in order to strengthen it. Reduction of its expression under the effect of the raw extract of *Aphanizomenon*[[-]] *flos-aquae* var. *flos-aquae* is consistent with the development of expressions of filaggrins and CLSP.

Please replace the paragraph beginning at page 11, line 3, with the following amended paragraph:

On the other hand, expression of calgranulins A and B, which are synthesized by the epithelial cells and keratinocytes, is increased under the effect of the raw extract of *Aphanizomenon*[[-]] *flos-aquae* var. *flos-aquae*. Psoriasin which like calgranulin A and calgranulin B, belongs to the S100 protein family, and the expression of which is inducible by retinoids (Tavakkov A. et al., a retinoic acid-inducible skin-specific gene (TIS-1/psoriasin): molecular cloning and analysis of gene expression in human skin *in vivo* and cultured skin cells *in vitro*, Mol. Biol. Rep., 1994, 20(2), 75-83) in differentiating primary keratinocytes, has an expression which also increases under the effect of the treatment. The same applies to the increase of the expression of MMP3 which is known to be significantly increased under the effect of retinoids (Varani J. et al., Expression of serine proteases and metalloproteinases in organ-cultured human skin. Altered levels in the presence of retinoic acid and possible relationship to retinoid-induced loss of epidermal cohesion, Am. J. Pathol., 1994, 145, 561-573.

**Please replace the paragraph beginning at page 11, line 18, with the following amended paragraph:**

All these events - activation of the relative expression of the messengers calgranulin A, calgranulin B, psoriasin, metalloprotease 3 and inhibition of the expression of messengers filaggrin, loricrin, calmodulin-like skin protein - let us anticipate a retinoid-like action of the topical application of the raw extract of *Aphanizomenon*[[-]] *flos-aquae* var. *flos-aquae*. Further, the increase in the expression of the tissular inhibitor of metalloprotease 1 (TIMP1) assumes an anti-ageing effect during topical application of a cosmetic composition based on *Aphanizomenon*[[-]] *flos-aquae* var. *flos-aquae*.

**Please replace the paragraph beginning at page 11, line 26, with the following amended paragraph:**

A composition for after-sun care comprises:

A1*	Demineralized water	qs**100%
A2	<u>Tetrasodium EDTA known under the trade</u> <u>registrations Sequestrene</u> <u>SEQUESTRENE</u> <u>®NA4/CelonCELON®E/TrilonTRILON®B</u>	0.01%
B1	<u>A composition of methylparaben known under</u> <u>the trade registration Nipagin</u> <u>NIPAGIN®M/MethylMETHYL-POB</u>	0.05%
C1	<u>A composition of carbomer known under the</u> <u>trade registration CarbopolCARBOPOL®940</u>	15.00%
D1	Triethanolamine	0.5-1%
E1	Antimicrobial preservative	0.5-1%
F1	Silicone	1-2%
F2	Perfume	0.15%

G1 Aphanizomenon[-] flos-aquae var. flos-aquae 0.5-5%  
extract

In solution in sorbitol and water (i.e. 1-5% of dry  
extract of Aphanizomenon[-] flos-aquae var. flos-  
aquae)

(\*: each of the letters placed in front of a component  
represents a phase)

(\*\* qs: *quantum satis*)

Please replace the paragraph beginning at page 12,  
line 12, with the following amended paragraph:

A composition for anti-ageing care comprises:

A1*	<u>A composition of Tribehenin PEG-20 Esters</u>	4,0%
	known under the trade registration of <u>Emulium</u>	
	<u>EMULIUM® (Gattefossé)</u>	
A2	<u>A composition of cholesterol soluble in oil</u>	6,0%
	known under the trade registration of	
	<u>Amercel<sup>AMERCOL®</sup> (Amerchol)</u>	
A3	<u>A composition of Isopropyl Lanolate</u> known	2,0%
	under the trade registration <u>Amerlate</u>	
	<u>AMERLATE® (Amerchol)</u>	
A4	<u>A composition of mineral oil and prunus</u>	2,0%
	<u>americana (apricot) kernel oil and calendula</u>	
	<u>officinalis flower extract</u> known under the	

trade registration Oily calendula Végétol  
VÉGÉTOL® (Gattefossé)

A5	<u>A composition of caprylic/capric/succinic triglyceride and sesamum indicum (sesame) seed oil and triticum vulgare (wheat) germ oil and tocopheryl acetate known under the trade registration of LNST®98 (Lanatech)</u>	1.0%
B1	Demineralized water	qs 100%
B2	<u>A composition of carbomer known under the trade registration of Carbopol CARBOPOL® (BF Goodrich)</u>	10.0%
C	<u>A composition of PEG/PPG dimethicone known under the trade registration of Abil ABIL® (Goldschmidt)</u>	6.0%
D1	Demineralized water	5.0%
D2	Triethanolamine (Prolabo)	0.2%
E1	Antimicrobial preservative	0.5%
E2	Natural glycerin (Elf Atochem)	4.0%
F	<u>A composition of aluminum starch octenylsuccinate known under the trade registration of Fluidamid FLUIDAMID®DF125 (Roquette)</u>	4.0%
G	<u>Aphanizomenon [I-] flos-aquae var. flos-aquae</u>	0.5-5%

extract in solution in sorbitol and water  
(i.e. 1-5% of dry *Aphanizomenon* [[-]] *flos-aquae* var. *flos-aquae* extract)

H Perfume 0.3%

Please replace the paragraph beginning at page 13,  
line 1, with the following amended paragraph:

A composition for washing and taking care of hair  
comprises:

A1*	<u>A composition of sodium lauryl sulfate</u>	10.00%
	<u>(anionic) known under the trade registration</u>	
	<u>of Texapon TEXAPON® (Henkel)</u>	
B1	Demineralized water	qs 100%
B2	<u>A composition of Tetrasodium EDTA known under</u>	0.05%
	<u>the trade registration of Sequestrene</u>	
	<u>SEQUESTRENE® (Prolabo)</u>	
C1	<u>A composition of cocamidopropyl betain known</u>	10.00%
	<u>under the trade registration of Tegebetain</u>	
	<u>TEGOBETAIN® (Goldschmidt)</u>	
D1	<u>A composition of fatty ester R(CO)OR'</u> known	4.00%
	<u>under the trade registration of Emilan EMILAN®</u>	
	<u>(Albright &amp; Wilson St Mihiel)</u>	
E1	<u>A composition of Glycerin and propylene</u>	3.00%
	<u>glycol and sorbitol and citrus grandis</u>	
	<u>(grapefruit) fruit extract and pyrus malus</u>	
	<u>(apple) fruit extract and prunus persica</u>	
	<u>(peach) fruit extract and sodium hyaluronate</u>	
	<u>known under the trade registration of</u>	
	<u>Hydralphatin HYDRALPHATIN®3P (Lanatech)</u>	
E2	Antimicrobial preservative	0.50%

F1	<u>A composition of cholesterol soluble in oil</u>	15.00%
	as as Amerchel AMERCHOL	
G1	<u>A composition of decyl glucoside known under</u>	6.00%
	<u>the trade registration of Oramix ORAMIX®</u>	
	(Seppic)	
G2	<u>A composition of ethoxylated stearic acid</u>	1.00%
	<u>known under the trade registration</u>	
	<u>Simulson SIMULSON® (Seppic)</u>	
H1	Demineralized water	5.00%
H2	<u>A composition of acrylic resin emulsions</u>	6.00%
	<u>known under the trade registration of</u>	
	<u>Acrylcel ACRYLSOL® (Seppic)</u>	
J1	<u>Aphanizomenon[[-]] flos-aquae var. flos-aquae</u>	0.5-5%
	extract in solution in sorbitol and water	
	(i.e. 1-5% of dry <u>Aphanizomenon[[-]] flos-</u>	
	<u>aquae var. flos-aquae extract)</u>	

Please replace the paragraph beginning at page 13,  
line 1, with the following amended paragraph:

An antiwrinkle care composition comprises:

A1*	<u>Demineralized water</u>	qs**100%
A2	<u>A composition of polyacrylamide/C1314/</u>	1.0%
	<u>Soparaffin/Laureth7- known under the trade</u>	
	<u>registration of Sepigel SEPIGEL® (Seppic)</u>	
B1	<u>A composition of Tribehenin PEG-20 Esters</u>	3.0%
	<u>known under the trade registration of Emulium</u>	
	<u>EMULIUM® (Gattefossé)</u>	
B2	<u>A composition of cholesterol soluble in oil</u>	4.0%
	as Amerchel AMERCHOL® (Amerchol)	

B3	<u>Isopropyl palmitate as Crodamol® (Croda)</u>	8.0%
B4	<u>A composition of PEG/PPG dimethicone known under the trade registration of Abil ABIL® (Goldschmidt)</u>	5.0%
C	Antimicrobial preservative	0.3%
D	<u>A composition of aluminum starch octenylsuccinate known under the trade registration of Fluidamid FLUIDAMID® DF15 (Gattefossé)</u>	3.0%
E	<u>Aphanizomenon[[—]] flos-aquae var. flos-aquae</u> 0.5-5% extract in solution in sorbitol and water (i.e. 1-5% of dry <u>Aphanizomenon[[—]] flos-aquae var. flos-aquae extract</u> )	

Please replace the paragraph beginning at page 14,  
line 5, with the following amended paragraph:

	A treatment mask composition for dried hair comprises:	
A1	<u>Cetearyl Glucoside (known under the trade registration of Montanov MONTANOV® 68-SEPPIC)</u>	7%
A2	<u>Coco betaine (known under the trade registration of AMONYL® 265BA-SEPPIC)</u>	0.5%
A3	Shea butter	4%
A4	Beeswax	2%
A5	Dimethicone (DOW CORNING)	5%
B1	Demineralized water	qs100%
B2	<u>Decyl glucoside (known under the trade registration of ORAMIX® NS10-SEPPIC)</u>	1%
C1	Perfume	0.5%
C2	Antimicrobial preservative	0.5%

C3 *Aphanizomenon*[-] *flos-aquae* var. *flos-aquae* 0.5-5%

extract in solution in sorbitol and water  
(i.e. 1-5% of dry *Aphanizomenon*[-] *flos-aquae* var. *flos-aquae* extract)

Please replace the paragraph beginning at page 14,  
line 20, with the following amended paragraph:

A night cream comprises:

A1\* Cetearyl glucoside (known under the trade 6%  
registration of Montanov MONTANOV® 68-SEPPIC)

A2 Vegetable oils 20%

A3 DL-alpha-tocopherol (BASF) 0.05%

B1 Demineralized water qs100%

C1 Antibacterial preservative 0.5%

C2 Perfume 0.3%

C4 *Aphanizomenon*[-] *flos-aquae* var. *flos-aquae* 0.5-5%  
extract in solution in sorbitol and water  
(i.e. 1-5% of dry *Aphanizomenon*[-] *flos-aquae* var. *flos-aquae* extract)